

A Broad Approach for the Management of Acute Promyelocytic Leukemia

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ABSTRACT

Acute promyelocytic leukemia (**APL**) is a blood cancer that affects people of all ages and strikes about 1,500 patients in the United States each year. For the past few decades, the standard treatment of APL has been based on the combined administration of all-trans retinoic acid (**ATRA**) and chemotherapy including anthracyclins and cytarabine. These combinations of therapeutic agents induce complete remissions (**CR**) in an average of 70% to 80% of adults aged less than 60 years. In 2009, U.S. Food and Drug Administration approved the use of arsenic trioxide (**ATO**) for the treatment of APL, based on clinical studies showing a complete remission, especially in relapsed patients. Recent reports show that patients with APL can be cured with ATRA and ATO combination, eliminating cytotoxic chemotherapy and resulting in superior outcomes compared to standard treatment. Despite advances and improvement of treatment outcome, APL remains associated with a significant incidence of early death. Early mortality in APL treated patients may reach up to 10% in clinical trials. The goal of the work presented in this book chapter is to provide not only an overview of APL, but also discuss potential risk factors that contribute to the development of APL and highlight major advances in the management of APL.

Keywords: Acute promyelocytic leukemia; All-trans retinoic acid; Arsenic trioxide; Risk factors; Treatment

INTRODUCTION

Acute promyelocytic leukemia (**APL**) is a blood cancer that affects people of all ages and strikes about 1,500 patients in the United States each year. It is a subset of acute myeloid leukemia (**AML**) defined by the formation of a chimeric gene, the promyelocytic leukemia-retinoic acid receptor α (*PML-RAR α*) [1]. APL is characterized by the t (15;17) (q22;q21.1). The t (15;17) translocation in APL is the result of two chromosome breaks: one in chromosome 15 and the other in chromosome 17 [2,3]. The signs and symptoms of APL are nonspecific and may include the following: fatigue or feeling tired, minor infections, tendency to bleed or hemorrhagic diathesis. There is usually pancytopenia with low levels of red blood cells or anemia, low levels of the white blood cells and platelets, dyspnea, fever, thrombocytopenia, leukocytosis and coagulopathy [4]. There is a variety of risk factors that contribute to the development of APL. It can affect anyone and many experts believe that APL is caused by a combination of environmental and genetic factors. Environmental exposure to high level of benzene has been linked to the development of APL. Benzene is currently one of the most documented risk factor for the development of leukemia [5]. Consumption of lipids in diet by European sea bass causes down regulation of *RAR α* and skeletal malformation during larval development [6]. The goal of the work presented in this book chapter is to provide not only an overview of APL, but also discuss potential risk factors that contribute to the development of APL and highlight major advances in the management of APL.

GENETIC AND ENVIRONMENTAL RISK FACTORS OF APL

Epidemiological feature of APL include constant incidence with age after age 20, equal incidence on male and females and higher frequency among patients originating in Latin America. The APL specific *PML/RAR α* gene re-arrangement is different in Latinos and non-Latinos [7]. Increased occurrence witnessed in adult patients of Latin America and Southern Europe is attributed to the altered APL-specific gene reorganization. A majority of breakpoints in the *RAR α* gene in the *PML/RAR α* transcript in intron 6 called bcr1 is believed to be genetically determined in APL [8]. A series of studies suggested that obesity and metabolic patterns of ingested vitamin A are associated with elevated risks of developing APL [9-11]. Epidemiological studies have shown an association between benzene exposure and incidence of acute myeloid leukemia (**AML**) [12, 13]. Ionizing radiations, chemotherapeutic agents such as alkylating agents and topoisomerase ii inhibitors have been reported to increase incidence of APL [7]. Another possible chemical has been reported in China, where exposure to bimatoprost, a psoriasis treatment drug was shown to be associated with subsequent AML, the most common subtype being APL [14]. Industrial applications of benzene and occupational exposure to benzene have been documented for workers in different production sectors including production of chemicals, pharmaceuticals, plastics, synthetic rubbers, and paints [15].

HALLMARKS OF ACUTE PROMYELOCYTIC LEUKEMIA

Acute promyelocytic leukemia (**APL**) is a unique subtype of the acute leukemias. It has distinct cytogenetics, clinical features, and biologic characteristics. It is caused by an arrest of leukocyte differentiation at the promyelocyte stage. Over 95% of cases are characterized by a balanced translocation between chromosome 17q21 and chromosome 15q22. This leads to an abnormal fusion protein called *PML/RAR α* . The translocation between chromosome 17q21 and chromosome 15q22 can be detected by karyotyping or fluorescence in situ hybridization (**FISH**) studies, and the transcript can be detected by polymerase chain reaction (**PCR**) techniques. The retinoic acid alpha receptor gene (*RAR α*) is encoded by the long arm of chromosome 17. It is mainly expressed in hematopoietic cells and has an important role in regulating gene expression. In the absence of retinoic acid, *RAR α* is bound by nuclear corepressor factor, and this causes transcriptional repression. In the presence of retinoic acid, *RAR α* is activated and terminal differentiation of promyelocytes occurs. The promyelocytic gene (*PML*) is encoded by the long arm of chromosome 15 and is expressed ubiquitously [16]. The *PML* gene is thought to be involved in apoptosis and tumor suppression, whereas the *RAR α* gene is mainly expressed in hematopoietic cells and has an important role in regulating gene expression related to myeloid differentiation [17]. Together, the *PML/RAR α* fusion prevents transcription of genes necessary for developing myeloid cells to differentiate past the promyelocyte differentiation stage. This results in abnormal promyelocytes proliferating and accumulating in the bone marrow and peripheral blood. The breakpoint in chromosome 17 is consistently found in intron 2, but varies in chromosome 15. The three breakpoints on the *PML* gene can occur at intron 3 (L form), intron 6 (S form), and exon 6 (V form). The S form is reportedly associated with a shorter remission duration and survival rate compared with the L form [18]. *PML/RAR α* is identified in 86%- 90% of patients with APL [19]. The *PML/RAR α* anomalous protein is believed to play a role in promyelocytic leukemogenesis [20]. Therefore, identification of *PML/RAR α* as a diagnostic marker in treatment of APL can enhance therapy [21].

TREATMENT OF APL BASED ON ATRA, ATO, AND COMBINATION OF ATRA PLUS ATO

Untreated acute promyelocytic leukemia is fatal and patients ultimately die of the leading complications associated with bone marrow failures namely due to infection and hemorrhage [22]. Historically, the treatment of APL was based on the administration of ATRA plus anthracycline chemotherapy, which is very effective as first line therapy; however, 25 to 30% of patients will relapse with their disease becoming refractory to conventional therapy. In recent years, ATO, which is used in Chinese traditional medicine, has been recommended for use as a potent antileukemic agent because of its ability to induce leukocytosis, and prolonged remissions even in retinoic acid (**RA**) resistant APL patients [23]. APL patients treated with ATO alone achieved high rate of 5-years disease free survival and an overall survival in Phase II clinical trial study

[24]. Previously, Ghavamzadeh and colleagues have shown that ATO induced complete remission with high survival rate without ATRA combination in APL patients [25]. Previous reports showed the beneficial combination of ATRA plus ATO in achieving a complete remission (**CR**) rate of 95% in low-risk patients and 79% in high-risk patients [26,27]. Another study by the North American Leukemia Intergroup Study C9710 evaluated the addition of ATO to consolidation with ATRA/chemotherapy and reported improved outcomes in the groups receiving ATO [29]. Today, the combination of ATRA and ATO represents the most successful treatment of all age group of APL patients with maximum survival rate [28]. The combination of ATRA plus ATO is now highly recommended upfront regimen for standard risk APL while previously it was recommended in the relapsed setting or in patients unable to receive anthracyclines [30]. Despite the most successful treatment of APL with ATRA and ATO, early death rates remain high due to hemorrhage complications, and infections which are the main cause of treatment failure in APL [31]. Several reports from multicenter cooperative studies suggested 5 to 10% early deaths within 1 month of treatment with APL patients [32-35]. Thus, new therapeutic strategies are needed to decrease early death rate and focus on survivorship care.

MECHANISMS OF ATRA AND/OR ATO COMBINATION IN THE TREATMENT OF APL

The mechanisms behind the effects of ATRA and/or ATO therapy for APL are poorly understood. Previous reports have shown that ATRA and ATO induce autophagy in leukemia cells through the mammalian target of rapamycin (mTOR) [36] or MEK/ERK pathway [37]. Additionally, Goussetis et al. (2010) showed that autophagic degradation leads to significant proteolysis of *PML-RAR α* [37]. These studies also showed that inhibitors of autophagy or molecular targeting of BECN1 or ATG7 results in reversal of the suppressive effects of ATO on leukemic cells [37]. Both ATO and ATRA induce leukemia-initiating cells (**LICs**) loss in *PML-RAR α* mouse APL and the combination of the two agents results in synergistic clearance of LICs through cooperative *PML-RAR α* degradation [38,39]. ATRA treatment is able to increase the expression of the cell membrane arsenic transporter AQP9, which facilitates arsenic uptake [40]. Furthermore, ATRA potentiates ATO-induced RXR α phosphorylation and cooperates with ATO to induce apoptosis [41]. A previous study indicated ATO represses NF-kappaB activation and increases apoptosis in ATRA-treated APL cells. [42]. ATRA plus ATO combinations lead to activation of ubiquitin-proteasome pathway and repressed genes/proteins promoting cell cycle of promyelocytic leukemia [43]. Many studies on APL-derived cell lines and transgenic mice carrying the *PML/RAR α* fusion proteins indicated that ATO induces degradation of both *PML/RAR α* and native PML from the nuclei of the malignant cells [44,45]. This allows partial differentiation of leukemia population to proceed [45]. As shown in Figure 1, a series of scientific reports in our laboratory demonstrated that the apoptotic mechanism of ATO as an anti-cancer drug for APL patients may be associated with DNA damage and cell death [46], up-regulation of *p53* tumor suppressor protein and repression of the *c-fos* transcription factor

[47] as result of oxidative stress [48], induction of phosphatidylserine externalization, caspase-3 activation, and nucleosomal DNA fragmentation [49]. Using differentiated APL cells, Ding and colleagues demonstrated that ATO directly binds on cysteine residues of *PML/RAR α* and small ubiquitin modifier (**SUMO**), which inhibit conjugating enzyme UBC9 and SUMO lyation, denature *PML*, activate p53 and reduce MDM2 [50]. Indirectly, ATO induces apoptosis by impairing H2O2 catabolism with a resultant decrease in mitochondrial membrane potential, release of cytochrome c, activation, and upregulation of caspases 1, 2, 3 and 8 [51, 52].

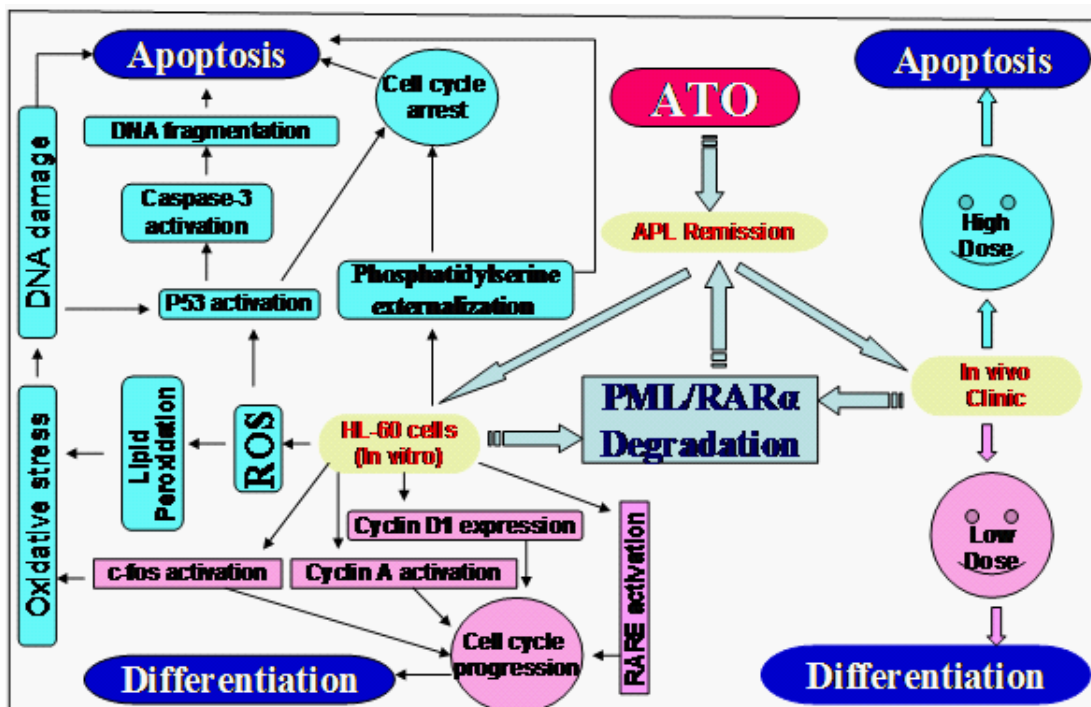


Figure 1: Schematic representation of the apoptotic mechanisms of ATO as a therapeutic agent in the treatment of acute promyelocytic leukemia. ATO exerts a dual effect on HL-60 cells by inducing partial differentiation and apoptosis [49].

CONCLUSIONS

APL accounts for about 10 to 15 percent of AML cases. The major goal of this review was to provide not only an overview of APL, but also discuss potential risk factors that contribute to the development of APL and highlight major advances in the management of APL. It is associated with a very high risk of severe bleeding complications, including early death from bleeding into the brain. ATRA or ATO is effective as first-line treatment for APL. The combination of ATRA plus ATO is now highly recommended upfront regimen for standard risk APL. The medical use of ATRA plus ATO in the management of APL patients has transformed the complete remission rate from a highly

fatal disease to a highly curable one. However, despite the most successful treatment of APL with ATRA plus ATO, early death rates remain high due to hemorrhage complications, and infections. Hence, new therapeutic approaches are needed to reduce or eliminate these early deaths. One of such therapeutic approaches is to test clinically the combination of ascorbic acid known as vitamin C (a natural supplement in our diet) and ATO for APL patients. A 2009 *in vitro* study in our laboratory demonstrated that ascorbic acid potentiated ATO anticancer activity against APL [53]. We found that ascorbic acid and ATO co-treatment exerts dual effects on human acute promyelocytic leukemia (**HL-60**) cells by inducing oxidative stress and subsequent inhibition of cell growth and induction of apoptosis [53]. Because ascorbic acid potentiates ATO anticancer activity *in vitro*, it would be interesting to carry further investigation to find out whether the combination of ascorbic acid and ATO is effective in the treatment of patients with APL.

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AUTHOR CONTRIBUTIONS

CGY, MS, PK, ATM, and DS conceived, designed, drafted the manuscript, and participated in the implementation of the study. FNK provided critical review and manuscript editing. PBT participated in the implementation of the study and manuscript review. All authors read and approved the final draft of the manuscript.

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